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Antimicrobial activity of *Salvia officinalis* acetone extract against pathogenic isolates

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ABSTRACT

Background & Aim: *Salvia officinalis* is known as a traditional medicine for several diseases in countries like Lebanon, Syria, Jordan, etc. In addition, *S. officinalis* has been subjected to several studies in a try to specify its medicinal effects. This study aims to evaluate the antimicrobial activity of this plant. The aim of the present research was to study antibacterial effects of *S. officinalis* on some bacteria.

Experimental: Three solvent extracts (de-ionized distilled water, Acetone, and Ethanol) of the plant were investigated against *Bacillus anthracis, Bacillus cereus, Escherichia coli, and Staphylococcus aureus* by using disc diffusion method.

Results & Discussion: Ethanol extracts showed moderate antimicrobial effect, while acetone extracts showed the most powerful antimicrobial effect, especially acetone leaves extracts. However, the de-ionized distilled water extracts showed no antimicrobial activity against the bacteria tested. The results indicate the inhibitory effects of acetone extract of *S. officinalis* with MIC= 10 mg/ml for *B. anthrac*is and MIC=30 mg/ml for *S. aureus*. Gram-negative microorganisms presented larger sensitivity for the extracts. As a result, organic solvent extracts (especially acetone leaves extracts) of this plant can be used as natural antimicrobial product. Results are in the aid of the fact as the polarity of the solvent decreases the antimicrobial compounds, and hence activity of the extracts, increases.

Industrial and practical recommendations: *Salvia officinalis* extract can be used as ointment for wound treatment. Science this extract has activity against *B. anthracis* we can introduce a new drug against *B. anthracis.*

1. Introduction

The discovery of antimicrobial activity of plants and spices goes back to ancient times (Ayres et al., 1980). A lot of effort has been paid to use plants and spices to eliminate microorganisms since their resistance is increasing against traditional antibiotics (Kunin, 1993; Finch, 1998). In Lebanon, Syria, Palestine, Jordan, etc. *Salvia officinalis* is the compulsory ingredient in cooking recipes and for tea making and is known as a traditional medicine for several diseases. The popular name of this plant in these countries is meramia. The Latin name *Salvia* comes from the Latin verb "salvare" which means to save, to heal. *Officinalis* in Latin means medicinal. The mere fact that both Latin names are referring to the medicinal properties, which cannot be said about any other plant, shows how much the ancient Romans appreciated sage two thousand years ago, and used it for healing in various ways (Dordević *et al.*, 2000). In this study, we investigate the

antibacterial activity of de-ionized distilled water, ethanol (70%), and aqueous acetone extracts of *Salvia officinalis* against pathogenic isolates and two fungi.

2. Materials and Methods

2.1. Plant material

Aerial parts of *S.officinalis* were collected from Khalda (Beirut, Lebanon). The plants were identified in the Department of Biology, Faculty of Sciences, Shahid Chamran University (Ahvaz, Iran), where a voucher specimen of the plant has been deposited. The plant was dried in shade, leaves were separated from stems, and then each one was pulverized separately by a mechanical grinder and stored in glass containers in dark until extraction.

2.2. Preparation of extracts

For extraction, de-ionized distilled water, ethanol (70%), and aqueous acetone were used as solvents. The ratio of each of leaves and stems powder to the solvents used are 5g/100ml for de-ionized distilled water, 5g/20ml for acetone, and 5g/50ml for ethanol (70%). The powder and solvent mixture were left in a conical flask for 72 hours, except the de-ionized distilled water extract which was first boiled for 20 minutes with continuous stirring then left in a conical flask for 72 hours. After 72 hours, each of the extracts was filtered through Whatman No.1 filter paper. The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness.

2.3. Bacterial cultures

One Gram negative bacteria *Escherichia coli* (XL blue1), and three Gram positive bacteria including *Bacillus anthracis* (pathogenic isolate), *Bacillus cereus* (pathogenic isolate) and *Staphylococcus aureus* (pathogenic isolate) were used in the study.

2.4. Antibacterial assay

The antibacterial activities of the extracts were investigated by the diffusion disc method (Veličković *et al*., 2003). The residual extracts were dissolved in their extracting solvents to yield a final concentration of 50 mg/ml and sterilized by filtration with filter pore size 0.45µm (Durmaz *et al*., 2006). Sterile filter paper discs (Whatman No.3, diameter 5mm) were impregnated with 50 µl of the prepared extracts. For the preparation of the inoculation, the tested bacteria were cultured in tryptone soya broth at 37°C for 24 h and standardized for the same absorbency, number 0.5 of the McFarland Nephelometer, which corresponds to the order of 10^8 CFU/ml (Durmaz *et al*., 2006). One hundred microliters of prepared culture were spread on the surface of Muller-Hinton agar. Previously prepared extract impregnated discs (Whatman No.1, diameter 5mm) were placed on the culture medium (Durmaz *et al*., 2006). The plates were incubated at 37°C for 24 h. Discs impregnated with solvents only were used as negatives. The antibacterial activity was evaluated by measuring the diameter of inhibition zone (Durmaz *et al*., 2006).

2.5. Determination of minimum inhibitory concentration (MIC) of leaves acetone extract

Cultures of the bacteria *Staphylococcus aureus* and *Bacillus anthracis* were diluted with a sterile physiologic saline solution [1% (w/v) sodium chloride] with reference to the 0.5 McFarland standards to achieve inoculums of approximately 10^8 colony forming units (CFU) per milliliter. Then, 2 ml of nutrient broth was added and a loopful of the test organism previously diluted was introduced to the tubes. Tubes containing bacterial cultures were then incubated at 37° C for 24 h. After incubation the tubes were then examined for microbial growth by preparing slides from each tube and observing them under the microscope (Velickovic *et al*., 2003, Razmavar *et al*., 2014).

2.6. Determination of Minimum Bactericidal Concentration (MBC) for leaves Acetone extract

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which didn't show any growth and inoculated on sterile nutrient agar. Plates inoculated with bacteria were then incubated at 37° C for 24 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration (Razmavar *et al*., 2014).

3. Results and discussion

In the study, among the extractions assayed, the acetone extracts of *Salvia officinalis* leaves contained the strongest antibacterial activity. Solvents, i.e. negative controls, used for extraction showed no

activity against any bacteria tested. Acetone and ethanol (70%) extracts produced inhibition zones against, both, Gram positive *B. anthracis*, *B. cereus* and *S. aureus*, as well as against Gram negative bacteria *E. coli*. While the water extracts, extracted from leaves and stems, failed to produce any inhibition zones. The present study was designed to obtain preliminary information on the antimicrobial activity of three *S. officinalis* extracts. The disc diffusion method was preferred in this study. Our study showed a remarkable antibacterial activity of the acetone extract of *S. officinalis.* Some study (Mosfa, *et al.*, 2013) on the ethanolic extract approved Gram-positive microorganisms presented larger sensitivity for the extracts. In contrast, our study showed Gram-negative microorganisms presented larger sensitivity for the extracts.

Table 1. Antibacterial activity of the various extracts from leaves and stems of *S. officinalis*.

Tested Microorganism	Inhibition zone diameters (mm) produced by extracts [a]							
	A-L	A-S	A-B	E-L	E-S	E-B	W-L	W-S
Escherichia coli	11	11	-	9	7	-	-	-
Staphylococcus aureus	17	12	-	10	7	-	-	-
Bacillus anthracis	25	21	-	17	12	-	-	-
Bacillus cereus	14	12	-	8	7	-	-	-

[a] Extracts: A-L, leaves acetone extract; A-S, stems acetone extract; A-B, blank acetone; E-L, leaves ethanol extract; E-S, stems ethanol extract; E-B, blank ethanol; W-L, leaves water extract; W-S, stems water extract.

Table 2. MIC and MBC of *S. officinalis* leave acetone extracts.

Tested Microorganism	Leaves Acetone extract	
	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	35	40
Bacillus anthracis	10	5

The inhibition zones varied depending on the type of extract and plant organ. The extracts of leaves and stems were more effective than other extracts where the largest inhibition zone was observed from acetone extracts of leaves against *B. anthracis.* Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The exceptional water-soluble compounds, such as polysaccharides and polypeptides, including fabatin and various lectins, are commonly more effective as inhibitors of pathogen adsorption and would not be identified in the screening techniques commonly used. Occasionally tannins and terpenoids will be found in the aqueous phase, which have antimicrobial effects through membrane disruption and enzyme inhibition, and are more often obtained by treatment with less polar solvents (Cowan, 1999; Shaik, *et al.*, 2014). Hence, since acetone has a polarity less than that of de-ionized distilled water and ethanol, it will contain more terpenoids and tannins, and will have a stronger antimicrobial effect. Our study results indicated that acetone extracts harbors a stronger antimicrobial activity than the other two extracts (Tirupatirao *et al.*, 2014).

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